Expression analysis of ROS scavenging enzyme encoding genes in rubber tree infected by *Microcyclus ulei*

Koop, D.M.[1]; Rio, M.[2]; Sabau, X.[2];Cardoso, S.E.A.[3]; Fernandes, V.[4];Leclercq, J.[2]; Garcia, D.[2]

[1]UESC,CBG, BA; [2]CIRAD, Dept. BIOS/UMR-AGAP, Montpellier, France; [3]Michelin Plantation, Igrapiúna, BA; [4]UESC, CME, BA

INTRODUCTION

South American Leaf Blight (SALB), caused by the ascomycete *Microcyclus ulei* is responsible for the low productivity of rubber tree in Latin America and represents an important threat for rubber plantations in Asia and Africa, where production is derived from highly susceptible clones. Reactive oxygen species (ROS) control many different processes in plants. In biotic interactions, ROS were proposed to orchestrate the establishment of plant defenses and hypersensitive response (HR) following successful pathogen recognition (Lamb and Dixon 1997). In this study, we present the expression of 9 genes potentially involved in ROS scavenging during infection of three different SALB resistant genotypes.

RESULTS

Expression of ROS scavenging encoding gene

The basal levels of expression of ***HbCat***, ***HbGCL1*** and ***HbGCL2*** were different between the genotypes (**Table 1**): in MDF 180, the relative transcript levels of *HbCat*, *HbGCL1* and *HbGCL2* genes were respectively 55-fold and 2- to 3-fold lower than in FX 2784 and PB 314 (**Figure 1**). Slight differences in basal levels of expression between genotypes were detected 24 and/or 48 hpi for *HbCuZnSOD*, *HbAPX1*, *HbAPX2,HbOASTL* and *HbMDHAR.*

M. ulei infection did not induce major variations of expression levels (several non significant *P* values for the variable Inoculation). Only two significant interaction between the genotype and the inoculation (G*I) were found for *HbGCL1* gene, which was down-regulated in PB 314 infected genotype and *HbOAST*L gene which was up-regulated in infected MDF180, both at 48 hpi (**Table 1**).

ROS scavenging encoding gene and DNA fragmentation

TUNEL and histological analysis indicated the presence of TUNEL positive nuclei (**Figure 4F**) and tissues degradation (**Figure 5C**) only in PB314 168hai. This histological differences between the three genotypes were not directly related to the variation of expression of the 9 studied genes involved in ROS scavenging. Further gene expression analysis of the pathways using ROS as lignin biosynthesis pathway (Garcia et al. 1995, 1999; Sambugaro et al. 2004; Koop et al. 2011) are required.

MATERIAL AND METHODS

Figure 1: *Hevea* leaf of three genotypes infected by *M. ulei* 10 dpi. Fx 2784 (HR), MDF 180 (partial resistance), PB 314 (susceptible).

- Inoculated and non-inoculated leaves were colected 24, 48, 96, 168 and 216 hpi (3 replicates).
- Transcript abundance was quantified by qRT-PCR and are relative to *Hbactin*. RT-PCR cycling conditions : one denaturation cycle at 95°C for 5 min, followed by 45 amplification cycles (95°C for 20 s, 55°C for 15 s, and 72°C for 20s). PCR reaction mixtures : 2µL of cDNA diluted 50 times, 1 µL of 5 µM of each primer, and 3µL 2x SYBR green PCR master mix (LightCycler® 480 SYBR Green I Master, Roche Applied Sciences).

Table 1: Primer list of studied genes.

Gene	Description	Foward primer Reverse primer
HbCuZnSOD	copper zinc superoxide dismutase (cytosolic)	AGACACAACAAATGGCTGC TGAGTGAAGGTCTTGTAAC
HbMnSOD	manganese superoxide dismutase	CTTGGACAAAGAATTGAAGAAGC ATACACTTCACTTGCATACTTCC
HbCAT	catalase	TATAGATCCTGGGCACCTG GGTGGCATCATCTTCAAATG
HbAPX1	ascorbate peroxydase	TTACCGATCCTGTCTTCC CCATCAACAACCAAACCAC
HbAPX2	ascorbate peroxydase	TTACCGATCCTGTCTTCC ATCAACCAACCCACTGCC
HbMDHAR	monodehydro ascorbate reductase	AGCCCGAGAAAAATATGGTGGC TTCAGTTTGCCAGAATCTATCCAG
HbOASTL	cysteine synthase	CATCAAGCCGGGTGAGAGTGTC CATGCCCCTTGCTGGATCAG
HbGCL 1	gamma-glutamylcysteine synthetase (cytosolic)	CAAGGAAATTGGGTTCTTG TCCAAAAATTCAATGCAG
HbGCL 2	gamma-glutamylcysteine synthetase (chloroplastid)	ACTCCTGAAGAAACACAAATGCTG GCCTCAGCAATCTAATTCACCTTAATAG
Hbactin	actin (housekeeping gene)	AGTGTGATGTGGATATCAGG GGGATGCAAGGATAGATC

Table 2: ANOVA of gene expression data testing the effect of genotype (G), inoculation (I) and interaction between the two parameters (G*I). Only *P* <0.05 are indicated. ns: non significant.

Gene	24 hpi			48 hpi			96 hpi			168 hpi			216 hpi		
	G	I	G*I	G	I	G*I	G	I	G*I	G	I	G*I	G	I	G*I
HbCuZnSOD	0.004	ns	ns	0 .028	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
HbMnSOD	ns	ns	ns	0.011	ns	ns	ns	ns	ns	ns	ns	ns	0.021	ns	ns
HbCAT	<0.0001	ns	ns	<0.0001	ns	ns	<0.0001	ns	ns	0 .004	ns	ns	0.009	ns	ns
HbAPX1	0.004	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
HbAPX2	0.011	ns	ns	ns	ns	ns	ns	ns	ns	0.029	ns	ns	ns	ns	ns
HbMDHAR	<0.0001	ns	ns	0.003	0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
HbOASTL	ns	ns	ns	0.009	ns	0.023	ns	ns	ns	ns	ns	ns	ns	ns	ns
HbGCL 1	<0.0001	ns	ns	<0.0001	ns	0.002	<0.0001	ns	ns	<0.0001	ns	ns	ns	ns	ns
HbGCL 2	<0.001	ns	ns	0.002	ns	ns	0.007	ns	ns	0.04	ns	ns	ns	ns	ns

Figure 2: Relative transcript levels of *HbCAT*, *HbGCL1*, *HbGCL2* genes in non-inoculated (□) and inoculated (■) leaves by *M. ulei* for three *Hevea* genotypes with different resistances. The same letter indicate similar values (Duncan test compare genotypes for the same time).

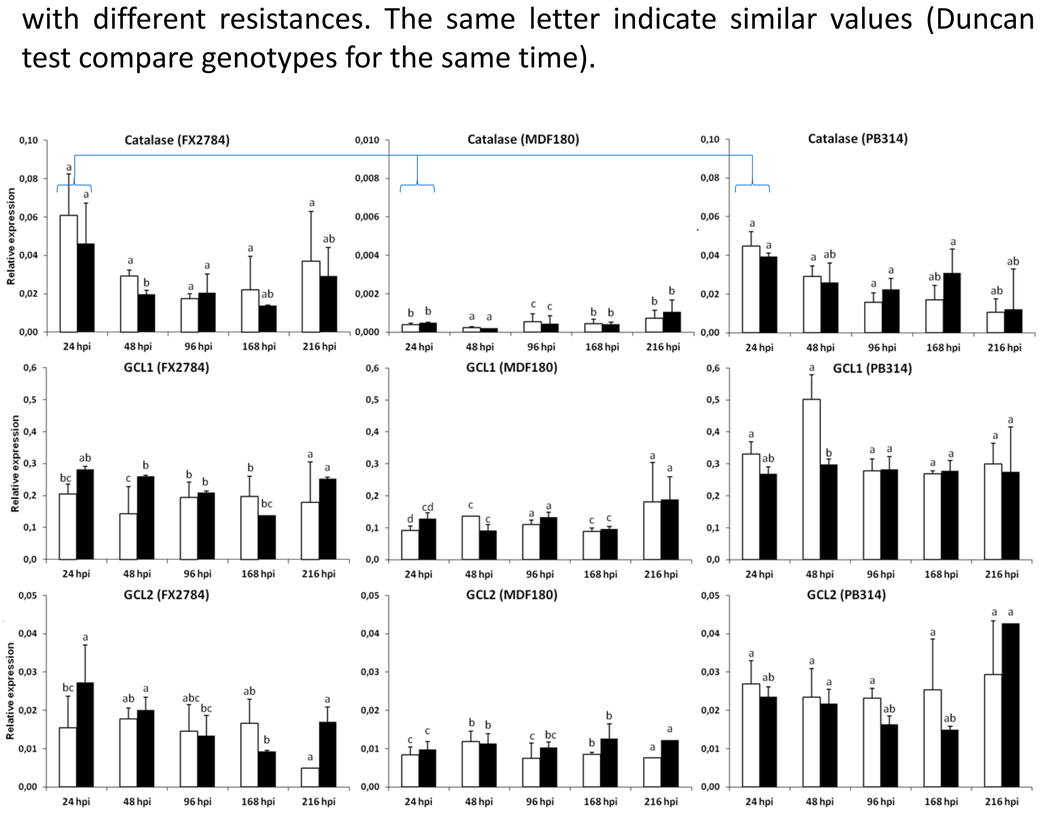


Figure 3: ROS scavenging pathway and potential key gene regulations in three *Hevea* genotypes.

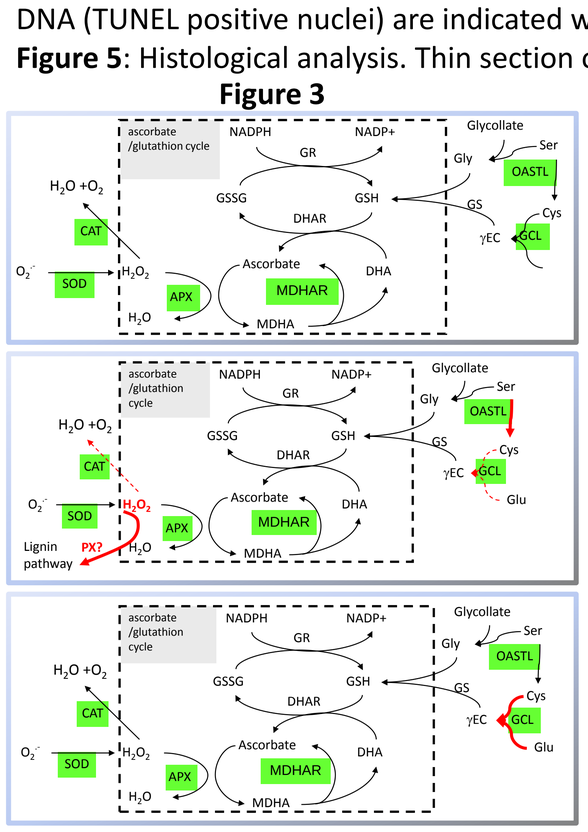


Figure 4: Analysis of thin sections of FX 2784, MDF 180 and PB 314 using the TUNEL method. Fragmented DNA (TUNEL positive nuclei) are indicated with white arrows (Koop, *et al* 2011). Bar = 20 µm.

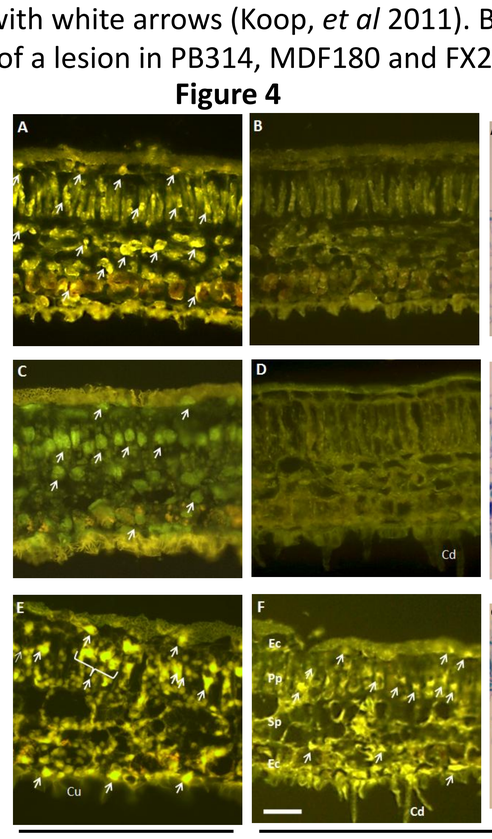
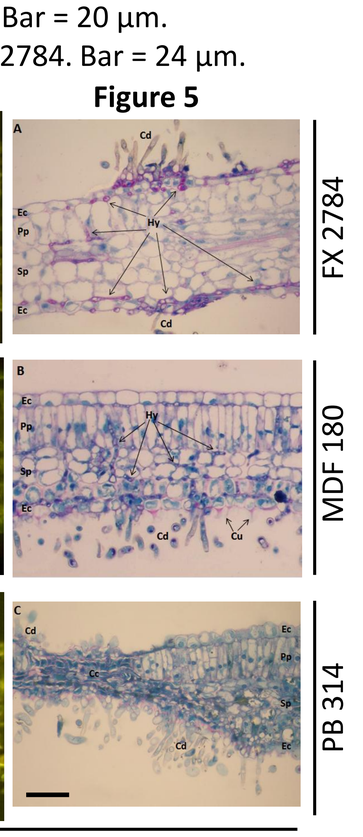


Figure 5: Histological analysis. Thin section of a lesion in PB314, MDF180 and FX2784. Bar = 24 µm.



Control (DNAse) — Inoculated leaf (168hpi)

CONCLUSIONS

- *HbCat*, *HbGCL1* and *HbGCL2* can be considered as reporter genes of the basal level of the oxidative status characteristic of the genotype;
- Other genes of the ROS scavenging pathway and lignin biosynthesis pathway have to be studied to get a best comprehensive overview of the oxidative status of the leaf and the possible variation of expression during infection by *M. ulei*.

Apoio Financeiro

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